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EFFECT OF Y-ORGAN ABLATION ON OOCYTE GROWTH IN THE TERRESTRIAL ISOPOD, *ARMADILLIDIUM VULGARE*

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Abstract

The reproductive and molting cycles of the isopod, *Armadillidium vulgare,* are synchronous during its breeding season. It was found that the rapid growth of oocytes occurs at stage D of the molting cycle. In hibernating females, the oocytes ceased to grow if their Y-organs were surgically extirpated at stage C in early spring. In these animals, many oocytes devoid of follicle cells degenerated along the inner margin of the ovary. Occasionally, however, a mass of granular substance was also seen in that part of the ovary. These results suggest that in *A. vulgare* the rapid growth of oocytes in stage D females is closely associated with activity of the Y-organ.

Introduction

In the terrestrial isopod *Armadillidium vulgare* as in many other species of Crustacea (Meusy and Charniaux-Cotton, 1984; Charniaux-Cotton, 1985), the reproductive and molting cycles are synchronous (Nakatsuchi, 1983). This suggests that the Y-organ, which is responsible for production of the molting hormone, may influence reproduction.

Cauterization of the Y-organs of the amphipod *Orchestia gammarella* inhibited further growth of oocytes and vitellogenin synthesis (Meusy *et al.,* 1977; Mathieu-Capderou, 1980; Blanchet-Tournier, 1982). In the isopods *Idotea balthica* (Reidenbach, 1971) and *Porcellio dilatatus* (Souty *et al.,* 1982), as well, cauterization of Y-organs resulted in arrested oocyte growth and lowered the rate of vitellogen release into the hemolymph. This method of cauterization of the Y-organs may produce some degenerated substances, which in turn exert an unfavorable influence upon the animal. A more desirable method is to ablate Y-organs by surgical extirpation.

The present study was undertaken to ascertain if surgical extirpation of the Y-organs arrests the growth of oocytes in *Armadillidium.* As observed under a dissecting microscope, the ovary of this species is a paired organ which extends longitudinally on both sides of the animal's cavity and is of modest size.

Materials and Methods

Animals

Specimens of terrestrial isopod, *Armadillidium vulgare,* used in this investigation were collected in February and March around Yokohama when the field population was in hibernation. Only adult females, 10–12 mm in body length, were used. Collected females were divided into the following five groups: initial specimens (Group A), animals with sham-operated Y-organs (Groups B and C), and animals with ablated

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Y-organs (Groups D and E). After operation, several females were kept in each petri dish with two adult males. Males and females were together for mating and oviposition by the females. The dishes containing moistened soil were placed in the laboratory at 25 ± 2°C and in natural daylight. Animals were fed decayed leaves and rat chows. Operations and observations were performed under a dissecting microscope. Surgical ablation of Y-organs was performed within a few days following collection. Animals were dissected after 25 (Groups B and D) and 50 (Groups C and E) post-operative days. Control animals (Group A) were similarly killed on the day the experiment started. The oocyte diameter was measured in ten largest oocytes of fresh ovaries in each animal.

To examine the interrelation between normal oocyte growth and the molting cycle, about fifty females were similarly selected and reared. They were sacrificed at three day intervals for observations on the development of the ovaries. Their fresh ovaries were observed with a light microscope.

The paraffin-imbedded ovaries of some females were sectioned 10 μm thick and stained with Mayer's hematoxylin and eosin.

Surgical ablation of Y-organs

The Y-organs of *A. vulgare* are bilaterally located at the base of the maxillary segment (Figs. 1A–D). The molt-controlling function of this organ has already been demonstrated by extirpation and reimplantation experiments (Suzuki, 1983).

A few days after collection, Y-organs were pulled from the females through a small hole using a pair of fine forceps. Operations were performed without any other treatment to the wounds. In a sham-operated animal, Y-organs were pulled out with forceps but then immediately placed back on their original sites. Success of the operation of the Y-organ ablation was ascertained by histologically studying the animals at the end of the experiment. As a result, it was verified that the Y-organs had been perfectly extirpated in all animals of Groups D and E.

Results

Observation concerning normal oocyte growth

The collected animals were at stage C of the molting cycle, had no sternolith on the thoracic segments (Madhavan, 1981), and had a pair of ovaries containing about one hundred oocytes (50–200 μm diameter) in each ovary (Fig. 2A and Group A in Table I). Larger oocytes (opaque yellow in fresh ovary) were located along the inner margin of the ovaries. Gradually the oocytes grew and were surrounded by a single layer of follicle cells. The sternoliths appeared about two weeks after collection, marking the beginning of stage D of the molting cycle. At that time most oocytes were approximately the same size (about 300 μm diameter). Rapid oocyte growth occurred in animals at stage D. One week later all oocytes had grown synchronously to become 500 μm in diameter, and were bright yellow (Fig. 2B). Ecdysis occurred first in the posterior region of the body, and one day later in the anterior region. Females laid about 80 eggs (530–650 μm diameter) in a pouch formed by the oostegites within 24 h after the anterior ecdysis. Forty-eight hours after the first oviposition, young oocytes (50–100 μm diameter) were seen lined up along the outer margin of the ovary (Fig. 2C). The young oocytes gradually increased in size and moved toward the inner margin of the ovary. Laid eggs were kept in the oostegites of ovigerous females for 16–19 days before larvae were released. About a week after release of the larvae, the remaining young oocytes grew rapidly again for the next oviposition soon after the appearance

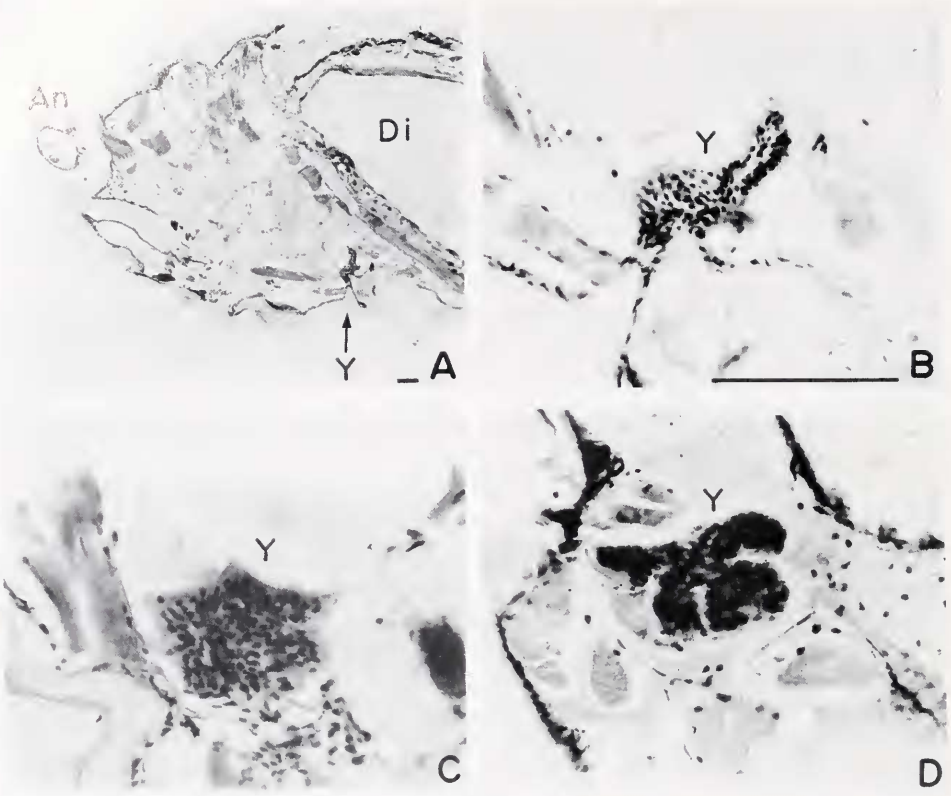

FIGURE 1. Location of Y-organs in vertical (A and B), horizontal (C) and transverse (D) sections of the head of *Armadillidium vulgare*. B is an enlargement of A through the Y-organ. Paraffin sections stained with hematoxylin-eosin. An, antenna; Di, digestive organ; Y, Y-organ. Scale bar = 100 μm.

of the sternoliths. During the experiment, almost all females laid the first and second eggs of the year in succession after each ecdysis.

Effect of Y-organ ablation on oocyte growth

Control females with Y-organs molted once (Groups B and C) or twice (Group C) during the experimental period (Table I), with their oocytes increasing in size. On the 25th day, about half the animals of Group B deposited eggs after molting and the other animals had large oocytes in their ovaries (Fig. 2D). On the 50th day, large oocytes for the secondary oviposition were also detected in the ovaries of Group C females. Some of them (30%) laid eggs in succession after the next molting.

No appreciable increase in oocyte diameter was noted during the experimental period in the Y-organ ectomized females (Groups D and E in Table I). The mean oocyte size in animals of Groups D and E was almost the same as in Group A, showing no increase in oocyte size in these animals. Moreover, exuviation and oviposition were completely blocked. On the 25th day following Y-organ ablation, most animals of Group D contained many oocytes, irregular in shape, along the inner margin of the ovary (Fig. 2E). On the 50th day, there were many traces of degenerating oocytes

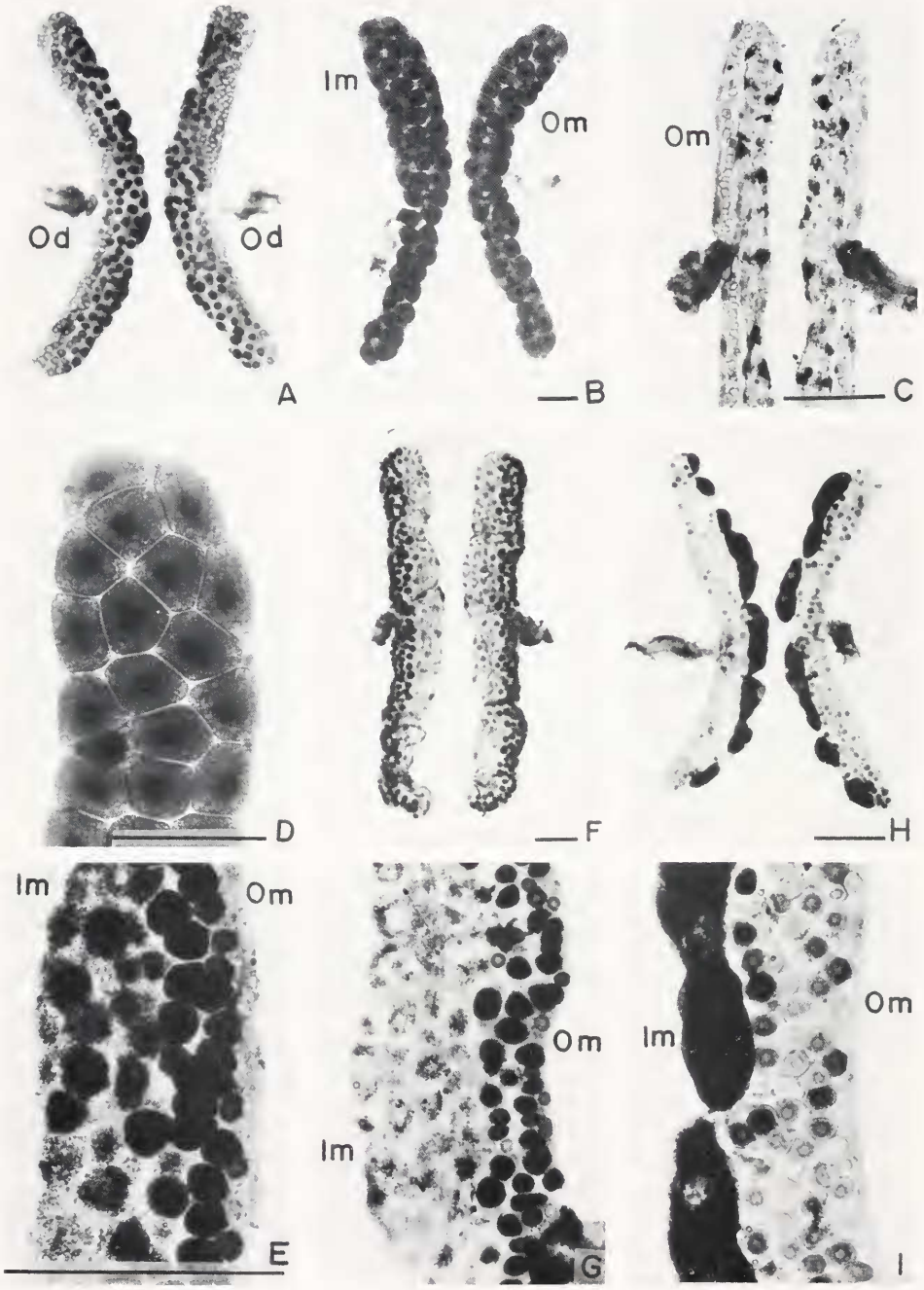

FIGURE 2. Photomicrographs of fresh ovaries of normal (A–D) and Y-organ ablated (E–I) females. A is 0 day, B, D, and E are 25 days, and F–I are 50 days after the beginning of the experiment. C represents the second day after oviposition. G and I are enlargements of F and H, respectively. Od, oviduct; Im, inner margin of the ovary; Om, outer margin of the ovary. Scale bar = 1 mm.

TABLE I

Effect of Y-organ ablation on oocyte growth of Armadillidium vulgare

Groups	Days after operation	No. of females	Oocyte diameter μm, mean ± S.D.	Percent of molted females	Percent of egg-laid females
A Initial specimens	0	15	184.8 ± 31.5	0	0
B Sham-operated	25	15	497.1 ± 125.5*	80.0	53.3
C Sham-operated	50	9	587.2 ± 31.2* 349.1 ± 203.2**	100	100
D Y-organs ablated	25	14	232.7 ± 31.6	0	0
E Y-organs ablated	50	12	203.5 ± 41.2	0	0

The oocyte diameter shows the mean size of ten largest oocytes of each animal at the time of experiment termination. When animal laid eggs during the experiment, it shows the mean diameter of ten laid eggs for the first (*) and second (**) oviposition.

in the inner margin of the ovary of many Group E animals (Figs. 2F, G) and follicle cells could not be observed around these degenerating oocytes. On the other hand, in some females of Group E (about 20%) a mass of granular substance (opaque yellow in fresh ovary and eosin positive) was observed along the inner margin of their ovaries (Figs. 2H, I). These ovaries (Fig. 2I) did not have as many degenerating oocytes as others of Group E (Fig. 2G). However, in the outer margin of the ovary of all Group D and E animals there were young and medium sized oocytes (Figs. 2E, G, I), with as normal an appearance as those of control females with Y-organs (Groups B and C).

DISCUSSION

In early spring *Armadillidium vulgare* females are in stage C of the molting cycle and have only young and medium size oocytes in their ovaries. The oocytes grow gradually for a while; rapid growth occurs abruptly when animals reach stage D. In this species, oocyte growth is coupled with the molting cycle; Nakatsuchi (1983) has already reported that the female reproductive cycle (vitellogenesis and oviposition) of this species is coupled with its molting cycle.

The present study shows that in the terrestrial isopod, *A. vulgare,* the presence of the Y-organ is needed for oocyte growth. The same result was already obtained in other isopods and amphipods as stated in the Introduction. The effect on isopod ovaries would be no different if the Y-organs were surgically extirpated or cauterized.

If surgical extirpation of the Y-organs was performed on *A. vulgare* at stage C, follicle cells would degenerate and oocytes would also degenerate in many animals (Figs. 2E, G). However, two different ovarian reactions were observed on the effect of Y-organ ablation, as shown in Figures 2G and I. These different reactions are left for further study.

Oviposition does not take place at every exuviation in *Armadillidium,* as shown in Table I (Group B). Other factors may exist for oocyte growth besides the molting hormone (Y-organ). In the female spider crab, *Libinia emarginata,* ovarian development occurs after the degeneration of the Y-organs (Hinsch, 1972). It is also pos-

tulated that a protocerebral factor is involved in the control of oocyte growth of the amphipod (Blanchet-Tournier *et al.,* 1980; Blanchet-Tournier, 1982). In the isopod, however, the protocerebrum appears to release a gonad-inhibiting hormone (Legrand *et al.,* 1982). It is possible that different Crustacea have developed various modes of hormonal control of the reproductive cycle (Meusy and Charniaux-Cotton, 1984; Charniaux-Cotton, 1985).

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LITERATURE CITED

BLANCHET-TOURNIER, M. F., J. J. MEUSY, AND H. JUNERA. 1980. Mue et vitellogenèse chez le Crustacé Amphipode *Orchestia gammarella* (Pallas). Étude des effects de la destruction de la région antéro-médiane du protocérébron sur la synthèse de la vitellogénine. *C. R. Acad. Sci. Paris* **291:** 829–832.

BLANCHET-TOURNIER, M. F. 1982. Quelques aspects des interactions hormonales entre la mue et la vitellogenèse chez le Crustacé Amphipode *Orchestia gammarellus* (Pallas). *Reprod. Nutr. Dev.* **22:** 325–344.

CHARNIAUX-COTTON, H. 1985. Vitellogenesis and its control in malacostracan Crustacea. *Am. Zool.* **25:** 197–206.

HINSCH, G. W. 1972. Some factors controlling reproduction in the spider crab, *Libinia emarginata. Biol. Bull.* **143:** 358–366.

LEGRAND, J. J., G. MARTIN, P. JUCHAULT, AND G. BESSE. 1982. Contrôle neuroendocrine de la reproduction chez les Crustacés. *J. Physiol. Paris* **78:** 543–552.

MADHAVAN, K. 1981. Induction of precocious molting in the male and ovigerous female *Armadillidium vulgare* by topical application of 20-hydroxyecdysone. *Gen. Comp. Endocrinol.* **44:** 28–36.

MATHIEU-CAPDEROU, C. 1980. Relation entre la maturation ovocytaire et l'exuviation chez le Crustacé Amphipode *Orchestia gammarellus* (Pallas). *C. R. Acad. Sci. Paris* **290:** 1495–1498.

MEUSY, J. J., M. F. BLANCHET, AND H. JUNERA. 1977. Mue et vitellogenèse chez le Crustacé Amphipode *Orchestia gammarella* Pallas. *Gen. Comp. Endocrinol.* **33:** 35–40.

MEUSY, J. J., AND H. CHARNIAUX-COTTON. 1984. Endocrine control of vitellogenesis in malacostraca Crustaceans. Pp. 231–241 in *Advances in Invertebrate Reproduction 3,* W. Engels *et al.,* ed. Elsevier Science Publishers, B. V.

NAKATSUCHI, Y. 1983. Change in the frequency of oogonial mitosis in *Armadillidium vulgare* (terrestrial isopod) during the reproductive season. *J. Fac. Sci. Univ. Tokyo Sec. IV.* **15:** 321–324.

REIDENBACH, J. M. 1971. Action d'une ecdysone de synthèse sur la mue et le fonctionnement ovarien chez le Crustacé Isopode *Idotea balthica* (Pallas). *C. R. Acad. Sci. Paris* **273:** 1614–1617.

SOUTY, C., G. BESSE, AND J. L. PICAUD. 1982. Stimulation par l'ecdysone du taux hemolymphatique de la vitellogénine chez le Crustacé Isopode terrestre *Porcellio dilatatus* Brandt. *C. R. Acad. Sci. Paris* **294:** 1057–1059.

SUZUKI, S. 1983. Effect of the Y-organ removal on the sexual organ in the isopod crustacean, *Armadillidium vulgare. Zool. Mag.* **92–4:** 574 (In Japanese).